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NEPHROLOGY FORUM

Making the diagnosis of Alport's syndrome

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CASE PRESENTATION

An 11-year-old girl was referred 10 years ago to the Cliniques Universitaires St. Luc for renal transplantation. At age 7, dipstick proteinuria and microscopic hematuria had been discovered at a school examination. Workup performed at that time at another hospital revealed a serum creatinine of 0.5 mg/dl, marked proteinuria (3.3 g/liter), and 100 red blood cells/high-power field on microscopy of the urine sediment; intravenous pyelography was normal. The patient's parents declined a renal biopsy. The patient was lost to follow-up for four years. She then presented at age 11 with end-stage renal failure (creatinine clearance, 2.6 ml/min/1.73 m^2). Ultrasonographic examination disclosed symmetrically reduced kidneys. Proteinuria and microscopic hematuria were again detected. Serologic tests, including complement studies, were unremarkable. There was no family history of renal disease. The patient's mother proposed donating a kidney to her daughter. The mother's assessment did not reveal any abnormality, and transplantation was performed two months after initiation of hemodialysis.

Post-transplantation immunosuppression included antilymphocyte globulin, cyclosporine, azathioprine, and prednisolone. The patient was discharged 13 days after surgery with a serum creatinine of 0.6 mg/dl. No rejection episode occurred. Histologic examination of the kidney removed at the time of transplantation showed on light microscopy mostly sclerotic, but also some normal-looking, glomeruli as well as diffuse interstitial scarring with a few inflammatory cells and foci of foam cells. Immunofluorescence studies disclosed only faint, focal staining for IgM, fibrinogen, and C3 along some vessel walls. No specific diagnosis was made.

Twenty months after transplantation, a hearing defect was detected at school. An audiogram revealed a symmetric loss of about 50 dB in the 1000 to 8000 Hz range, a loss typical of sensorineural deafness (Fig. 1). A diagnosis of Alport's syndrome (AS) was suspected. Ophthalmologic evaluation failed to show lenticonus and macular flecks. Electron microscopic examination of the nephrectomy specimen revealed a grossly abnormal appearance of the glomerular basement membrane (GBM) typical of Alport's syndrome, including irregular contours, areas of thinning, and marked thickening and splitting with inclusion of electron-dense granules in electron-lucent areas (Fig. 2).

Despite the absence of renal disease in this family, a clinical and genetic screening of the first relatives was proposed. The parents told us for the first time that they were first cousins; this information raised the suspicion of a recessive form of Alport's syndrome. Both parents and the patient's three siblings had normal urinalyses and normal serum creatinine levels. The father was mildly hypertensive and had a bilateral, symmetric hearing loss in the 3000 to 6000 Hz range, attributed to his work in a noisy environment. The mother and the three siblings had normal blood pressure and normal audiograms, except for a slight unilateral hearing loss in one sister.

Linkage analysis using six genetic markers near and within the COL4A5 gene, the gene responsible for the X-linked form of Alport's syndrome, showed that one unaffected brother had received from his mother a copy of the same haplotype as the proband. Thus the COL4A5 gene was not responsible for the disease in this family. In fact, both the consanguinity and the development of renal failure in a very young female strongly suggested a recessive form of Alport's syndrome involving the COL4A3 or COL4A4 gene. Direct sequencing of PCR products from the first five exons of COL4A3 (counted from the 3′ end of the gene) disclosed a homozygous single-base substitution of T to C in exon 5 of COL4A3; this mutation replaces an arginine codon with a stop codon, shortening the α3(IV) chain by 190 amino acids [1]. Further immunohistochemical study of the preserved glomeruli and tubules from the nephrectomy specimen revealed no fixation of anti-α3, -α4, and -α5(IV) antibodies in the GBM, whereas isolated staining of anti-α5(IV) was observed in the basement membranes of Bowman's capsules and collecting ducts (Fig. 3), a pattern consistent with the autosomal-recessive form of Alport's syndrome [2].

The patient was given information on the recessive nature of her disease. She subsequently became pregnant and gave birth one year ago to a normal girl. She is currently doing well 10 years after transplantation and has normal graft function. Her

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Key words: COL4A5 gene, hereditary nephritis, type-IV collagen, hematuria, proteinuria, end-stage renal failure, glomerular basement membrane, lenticonus.

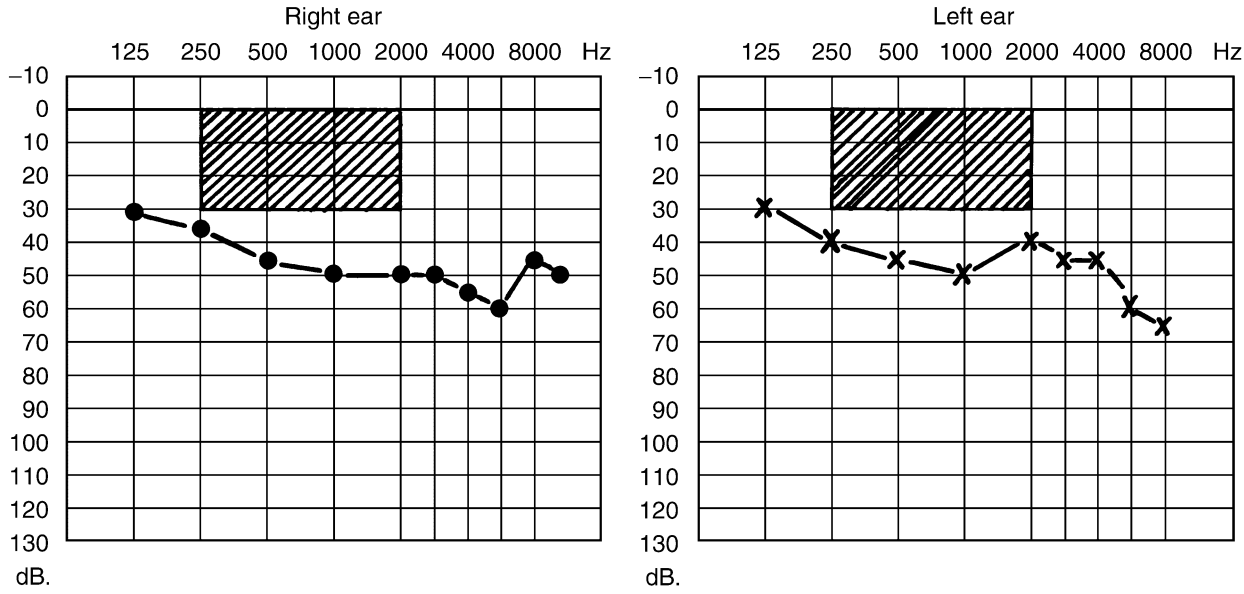

Fig. 1. Tonal audiometry showing a bilateral, symmetric loss (in decibels or dB) most marked in high tones (1000–8000 Hertz). Hatched rectangle is the area of conversational speech.

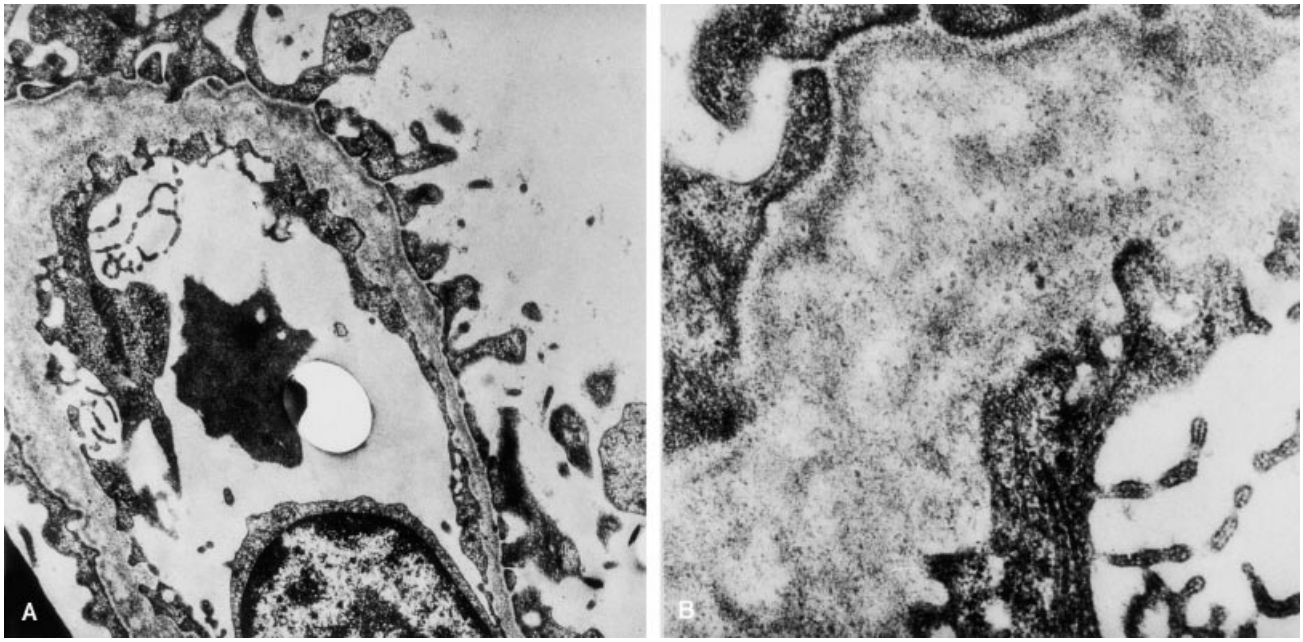

Fig. 2. Electron photomicrograph of glomerular basement membrane, showing segments of thickening and thinning with irregular contours (left panel). Magnification of a thickened segment showing lamellation, electron-lucent areas and electron-dense granules (right panel). Courtesy of Dr. J.P. Cosyns.

maintenance daily immunosuppressive regimen includes cyclosporine, 2 mg/kg; azathioprine, 1 mg/kg; and prednisolone, 0.1 mg/kg.

DISCUSSION

Dr. Yves Pirson (*Clinical Professor, Service de Néphrologie, Université Catholique de Louvain, Cliniques Universitaires Saint-Luc, Brussels, Belgium*): As the case presentation illustrates, the diagnosis of Alport's syndrome can escape a physician's attention in the absence of a family history of the disease. Furthermore, even after the recognition of both sensorineural deafness and GBM abnormalities, this case would not have met the strict diagnostic criteria proposed 10 years ago for "classic" AS [3], because both a family history and ophthalmic signs were absent. Since identification of the

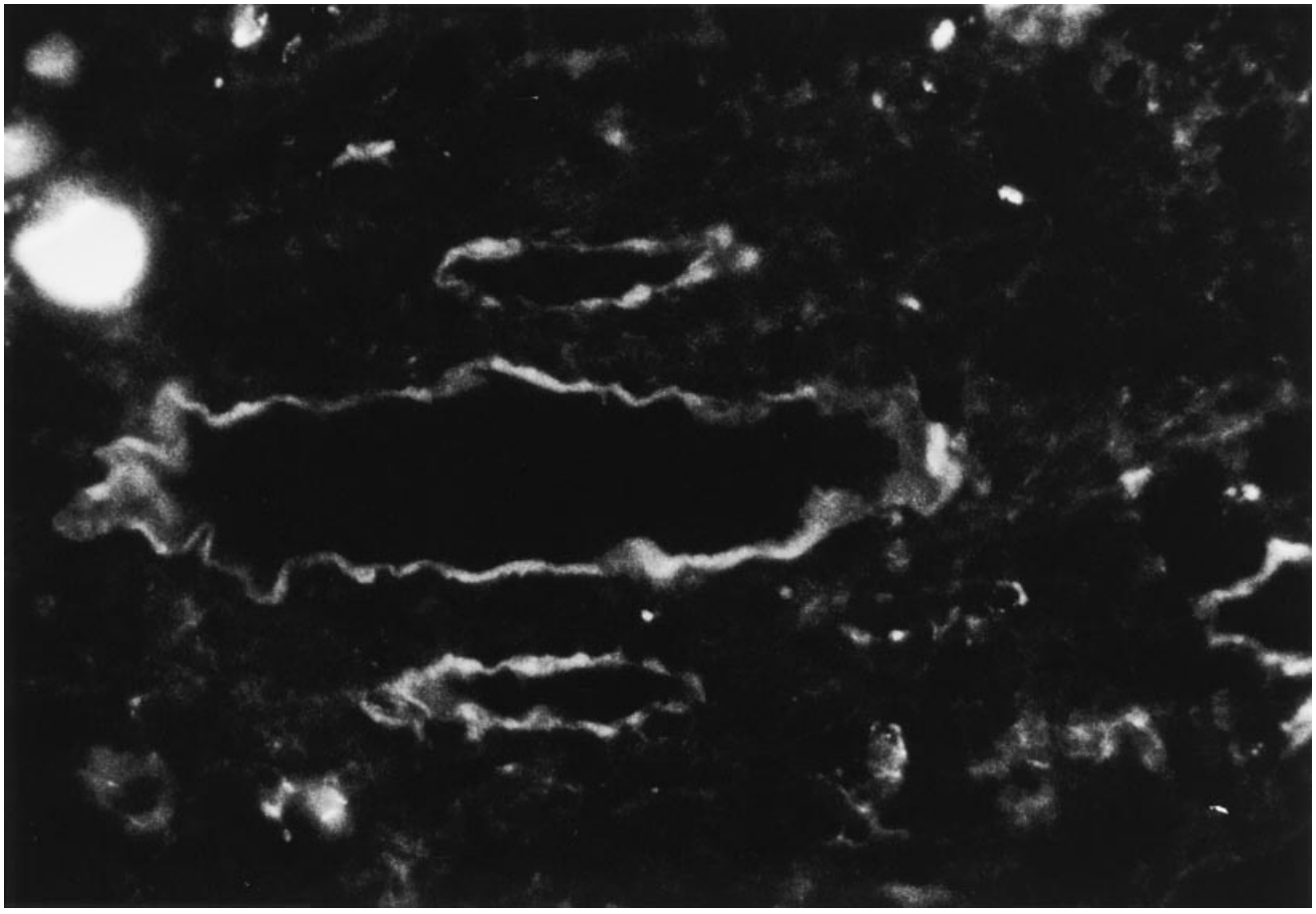

Fig. 3. Immunostaining of the nephrectomy spectrum with antiα5 (IV) antibodies, showing expression in basement membranes of collecting ducts. Courtesy of Dr. M.C. Gubler.

genes responsible for AS, first the "classic" X-linked in 1990 [4] and in 1994 the autosomal-recessive forms [1], genetic mutation detection has become the ultimate and sufficient diagnostic criterion. However, mutation analysis of the AS genes remains a tedious and sometimes unsuccessful task because the incriminated genes contain more than 50 exons and there is no "hot spot." The development of new DNA analyses such as the chip technology could improve the yield of mutation screening in the near future [5]. In the meantime, the diagnosis of AS rests in the majority of cases on a careful stepwise approach exemplified by the case presentation: careful collection and analysis of family history and clinical features, electron microscopic and immunohistologic examination of basement membranes, and genetic linkage analysis. After a brief review of the pathogenesis of the disease, I will critically review the diagnostic value of each clinical and pathologic feature and propose a diagnostic strategy.

Alport's syndrome is characterized by a progressive glomerulonephritis erratically associated with various extrarenal features, mainly auditory and ocular defects [6, 7]. Estimated gene frequency is 1:5000 to 1:10000; AS accounts for 1% to 2% of cases of end-stage renal failure (ESRF) in Western countries [8]. Alport's syndrome is caused by defects in type-IV collagen, a major component of basement membranes. Type-IV collagen is composed of three α-chains that form a triple helical molecule. Each α-chain consists of a central domain (the collagenous domain), an amino terminal end (the 7S domain), and a globular carboxy terminal end (the noncollagenous domain or NC1 domain) [9]. Six genetically distinct type-IV collagen α-chains, α1(IV) through α6(IV), have been identified. They are encoded by genes (called COL4A1 to A6) arranged pairwise on three different chromosomes: COL4A1 and COL4A2 on chromosome 13, COL4A3 and COL4A4 on chromosome 2, and COL4A5 and COL4A6 on chromosome X [10]. The α1(IV) and α2(IV) chains are present in all basement membranes; mutations in their genes are likely to be lethal in the embryo [10]. By contrast, the α3, α4, α5, and α6(IV) chains are expressed selectively in the basement membranes of some tissues, including those potentially involved in AS, that is, kidney, cochlea, and eye [11].

In approximately 85% of AS pedigrees, the disease is X-linked and mutations identified so far are in the COL4A5 gene. Of 176 mutations in the COL4A5 gene recorded by Lemmink and coworkers, 38 are large- and medium-sized deletions (ranging from a single exon to the complete gene) and 138 are small mutations of all types (mis-sense: 62; frameshift group including small deletion, insertion, splice site and non-sense: 69; inframe group including deletion and duplication: 7) [12]. The mutations are interspersed along the gene. New mutations comprise 10% to 15%, a proportion comparable with that found in other X-linked disorders [12]. In the few X-linked families with associated leiomyomatosis, Antignac and Heidet have identified contiguous deletions involving both the COL4A5 and the COL4A6 genes [13]. So far, no mutation solely within the COL4A6 gene has been reported in patients with AS.

In the majority of the non-X-linked families, the transmission appears to be autosomal recessive, with mutations detected in either the COL4A3 or the COL4A4 gene; Lemmink et al have recorded nine different mutations (six in COL4A3, three in COL4A4) [12]. Of interest, mutations in the COL4A3/A4 genes appear to be involved in a subset of patients with so-called benign familial hematuria [14] and perhaps also in a rare, autosomal-dominant, form of AS [15]. I will discuss these preliminary data briefly in a moment.

Clinical features

In the X-linked form of AS, the disease is consistently severe in affected males (hemizygotes) and usually much less symptomatic in affected females (referred to as XL heterozygotes); the wide range of severity observed in females is ascribed to the well-known random inactivation of one of the X chromosomes [16]. In the autosomal-recessive form, the disease is, as expected, as severe in male as in female homozygotes or in the rare compound heterozygotes, referred to collectively as AR homozygotes for the sake of simplicity; mild clinical manifestations are occasionally observed in carriers of the autosomal recessive form (referred to as AR heterozygotes).

Renal disease. Hematuria is the key clinical feature in AS. Just as in other glomerular diseases, examination of the urine frequently reveals red blood cell casts and, in phase-contrast microscopy, acanthocyturia [6]. In the X-linked form, microscopic hematuria is observed in all males and in 95% of females [17]. In hemizygotes, microscopic hematuria is constant from infancy, whereas it can be intermittent in XL heterozygotes. Episodes of gross hematuria occur in 60% to 70% of hemizygotes (sometimes after an upper respiratory tract infection, just as in IgA nephropathy), most often before the age of 15 [8], and in one-third of XL heterozygotes [8, 17]. In the autosomal-recessive form, all AR homozygotes have microscopic hematuria and many have episodes of gross hematuria; interestingly, microscopic hematuria is found in about one-half of AR heterozygotes [1, 12].

Proteinuria, usually absent in the first years of life, eventually appears in all hemizygotes and AR homozygotes and can lead to the nephrotic syndrome in as many as 30% of patients [6–8]. Proteinuria is recorded in two-thirds of XL heterozygotes [8].

Not unexpectedly, the risk of progression to end-stage renal failure is the highest among hemizygotes and AR homozygotes. In hemizygotes, the probability of developing ESRF is 90% by the age of 40 (abstract, Gubler et al, *J Am Soc Nephrol* 9:388A, 1998). According to the age at ESRF in hemizygotes, X-linked AS is said to be of either the juvenile or the adult type, the arbitrary cut-off being 31 years [18]. The juvenile type is encountered in three-quarters of kindreds. In XL heterozygotes, the probability of developing ESRF is 12% by the age of 40 and 30% by the age of 60 (abstract; Gubler et al, ibid); risk factors for progression are a history of gross hematuria in childhood, nephrotic syndrome, and diffuse GBM thickening [19].

Renal prognosis also depends on the kind of mutation. Among hemizygotes the probability of ESRF before the age of 30 is significantly higher (90%) in individuals with a large rearrangement of COL4A5 or a small mutation leading to a stop codon than in those with a splice-site (70%) or mis-sense (50%) mutation (abstract; Gubler et al, ibid). As a result, a good concordance usually exists for the age of onset of ESRF among affected relatives [8], especially in the juvenile type. Still, a large intrafamilial variability—as indicated by a more than 20-year difference in the age at ESRF in related hemizygotes—has been reported in the adult type [20–22]. A more than 10-year difference in the age at ESRF also has been observed among related AR homozygotes [1, 23].

Hearing defect. Sensorineural deafness used to be a prerequisite for the diagnosis of AS [3]. It is now clear that hearing is not impaired in some families with X-linked as well as with autosomal-recessive AS [1, 7, 12]. Hearing loss is never present at birth. Generally (but not always, as exemplified in the case presentation), hearing loss becomes symptomatic before the onset of renal failure. In the early stage, it is detectable only by audiometry, revealing a bilateral high tone loss (defined as -30 dB) most marked in the 2000 to 8000 Hz frequency range [7, 24]. Serial studies demonstrate a progressive loss of as much as 50 to 70 dB, as well as loss at lower frequencies [7, 24]. Hearing loss can affect conversational speech to the extent that patients require a hearing aid.

The risk of developing hearing loss by the age of 40 is about 90% for hemizygotes and 10% for XL heterozygotes (abstract; Gubler et al, ibid). Hearing loss is present in about two-thirds of AR homozygotes, usually before the age of 20 [12].

Table 1. Non-Alport hereditary disorders that may include renal involvement and hearing loss

Disease	Ref.	Renal involvement	Hearing deficit	Main other abnormalities	Genetics
Mitochondrial cytopathy	26, 27	Nonspecific tubular and glomerular lesions	Sensorineural	Diabetes, short stature, cardiomyopathy	A3243G mutation in mtDNA[a]
Muckle-Wells syndrome	28	AA amyloidosis	Sensorineural	Bouts of fever and angioedema-like symptoms	Unclear
Refsum's disease	8	Storage nephropathy	Sensorineural	Retinitis pigmentosa, peripheral neuropathy, cerebellar ataxia	AD, gene unknown
Cockayne's syndrome	29	Nonspecific glomerulosclerosis	Sensorineural	Photosensitivity, retinitis pigmentosa, poor growth	AR, gene unknown
Branchio-oto-renal syndrome	29	Agenesis and/or hypo/dysplasia ± cysts	Sensorineural, conductive or mixed	Preauricular pits, branchial fistulae, facial dysmorphy	AD, EYA1 gene (chrom 8)
Bardet-Biedl syndrome	29	Dysplasia	Sensorineural	Retinitis pigmentosa, obesity, polydactyly hypogenitalism in males	AR, loci at chrom 3, 11 15, 16
Alström's syndrome	29	Dysplasia	Sensorineural	Retinitis pigmentosa, obesity, polydactyly	AR, locus at chrom 2
Charcot-Marie-Tooth syndrome	30	Nonspecific glomerulosclerosis	Sensorineural	Slowly progressive neuromuscular atrophy	AD, gene unknown
Distal tubular acidosis	31	Metabolic acidosis ± nephrocalcinosis	Sensorineural	—	AR, gene unknown

[a] mtDNA: mitochondrial DNA; AD: autosomal dominant; AR: autosomal recessive

Just as for renal prognosis, hearing loss is determined by the kind of mutation: among hemizygotes the probability of developing hearing loss before the age of 30 is significantly lower in those with a mis-sense mutation than in those with other types of mutation (abstract; Gubler et al, ibid). Of note, a specific COL4A5 mis-sense mutation, found in 87 males from nine US families sharing a common ancestry, is associated with a remarkable late-onset hearing loss, reported in only 60% of patients by age 60, 10 years on average after ESRF [22]; interestingly, the age at which hearing loss becomes apparent is highly variable in this family (with an interval of as long as 30 years) [22].

Limited information is available on the histology of the inner ear in patients with AS. Data point to the stria vascularis of the cochlea as the site of the most striking alterations [7]. In a mouse model of AS, a marked thickening of the basement membranes of the strial vessels has been observed [25].

Sensorineural deafness developing in the presence of nephropathy should suggest several other hereditary disorders (Table 1) [9], which are rarer, and therefore less well known, than AS. A specific mitochondrial DNA (mtDNA) mutation (a guanine for adenine substitution at position 3243) is responsible for a syndrome combining diabetes mellitus, sensorineural deafness, and progressive renal failure [26, 27]; remarkably, several reported patients had been misdiagnosed as having AS [27]. The manifestations of the other listed diseases are clearly different from AS. Muckle-Wells syndrome is characterized by recurrent bouts of fever with urticaria and limb pain complicated by AA amyloidosis and the eventual development of the nephrotic syndrome [28]. Cockayne syndrome is characterized by a variety of clinical features including glomerulonephritis, photosensitivity, precocious senile appearance, neurologic abnormalities, and retinitis pigmentosa [29]. Various associations between malformed ears, hearing loss, and renal abnormalities have been described; the most common is the branchio-oto-renal syndrome [29]. Bardet-Biedl syndrome, as well as its variant, Alström's syndrome (in which hypogenitalism is absent), can include both renal cystic dysplasia and deafness [29]. A few patients with the Charcot-Marie-Tooth syndrome have focal segmental glomerulosclerosis associated with hearing loss [30]. Sensorineural deafness also has been reported in patients with the autosomal-recessive, but not the autosomal-dominant, form of distal tubular acidosis [31]. One family with autosomal-dominant hypoparathyroidism, sensorineural deafness, and renal dysplasia has been reported [32].

Ocular abnormalities. Ophthalmic abnormalities occur in about one-half of AS patients (Table 2). Reported ocular changes include anterior lenticonus and retinal flecks. In addition, we have described recurrent, non-traumatic corneal erosions [33]. Lenticonus is a localized conical (hence its name) protrusion of the central portion of the lens, most often into the anterior chamber of the eye. Anterior lenticonus was recorded in 22% of 94 AS patients younger than 25 years (Table 2). Anterior lenticonus, the most specific extrarenal manifestation of AS, is pathognomonic of AS [34]. It also is a valuable marker of the severity of AS, being consistently associated with

Table 2. Prevalence of main ocular abnormalities in 94 AS patients younger than 25 years old

Ref.	Number of patients/males	Number of patients with			
		Any ocular abnormality	Anterior lenticonus	Retinal flecks	Posterior polymorphous dystrophy
24	51/43	23	10	18	0
34	9/8	8	4	7	0
37	6/6	6	1	2	2
38	8/6	6	4	4	5
39	20/15	8	2	4	0
	94/78	51 (54%)	21 (22%)	35 (37%)	7 (7%)

both a rapid progression to renal failure and hearing loss [6–8]. The anterior lens surface is best examined with a slit lamp. At fundus examination, lenticonus appears as a central shadow in the red reflex, like an oil droplet on water. Lenticonus is never present at birth; it develops slowly during adolescence and can produce axial myopia and occasionally lens opacities or spontaneous rupture of the anterior capsule [6–8]. The latter complication is accounted for by the marked thinning of this capsule [35]. Lensectomy and intra-ocular lens replacement are sometimes required.

Macular flecks appear as bilateral, faint, whitish or yellowish dot-like lesions in the perifoveal area or mid-periphery of the retina [36]. They were present in 37% of the young patients reported in five series (Table 2) [24, 34, 37–39]. Although often associated with deafness and lenticonus, retinal flecks may be the only extrarenal manifestation of AS and thus constitute a valuable element in the diagnostic approach. They should not be confused with albipunctatus retinopathy seen in other disorders, including familial interstitial nephritis [40]. Absent during infancy, retinal flecks develop with time. They do not impair vision; electrophysiologic and fluoroangiographic evaluation yield normal results [8].

Recurrent corneal erosion has been observed in 23% of 44 patients with severe AS (all were deaf and 38 had reached end-stage renal failure) [33]. We found it interesting that the first episode of corneal erosion preceded the diagnosis of AS in three patients. This complication is characterized by attacks of acute ocular pain, photophobia, and tearing, a triad of symptoms that generally first occurs between the ages of 12 and 21 [33]. Episodes are triggered by outdoor activity during windy conditions, lack of sleep, long periods of ocular concentration, and use of contact lenses. Slit-lamp examination discloses localized abrasion of the corneal epithelium and occasionally a detached flap. Outside the erosion, the cornea appears normal or shows occasional intraepithelial microcysts [33]. A history of recurrent corneal erosion does not correlate with the presence of lenticonus or retinal flecks [33]. Endothelial vesicles compatible with posterior polymorphous dystrophy of the cornea, reported by two authors [37, 38] have not been observed by others (Table 2) [24, 34, 39].

The ocular abnormalities associated with AS are readily accounted for by the presence of type-IV collagen containing the α3, α4, and α5 chains in the basement membranes of the lens capsule, retina (Bruch's membrane and internal limiting membrane), and cornea (epithelial and endothelial) [11, 41–43]. Just as in the GBM, a structural defect in the α3, α4, or α5 chain might prevent or disturb their incorporation in the ocular basement membranes and thus increase their fragility in the lens, or corneal epithelium, or modify their appearance as retinal flecks or posterior polymorphous dystrophy.

Leiomyomatosis. In 35 patients from 19 families reviewed by Antignac and Heidet, a juvenile-type X-linked AS was associated with diffuse esophageal leiomyomatosis, mainly manifested by dysphagia or retrosternal pain [13]. Leiomyomatosis also can involve the tracheobronchial tree and female genitalia and is associated with congenital bilateral cataracts. Using molecular biologic methods, this same group found a deletion involving the two first exons of COL4A6 and a variable part of the contiguous 5′ end of COL4A5 [13]. Leiomyomatosis is as severe in females as in males, whereas renal involvement is milder in females, just as in X-linked forms without leiomyomatosis [44]. The role of the COL4A6 mutation in the development of this smooth-muscle-cell tumoral process remains to be elucidated.

Morphologic features

Study of the kidney by light microscopy and conventional immunofluorescence provides no specific clue to the diagnosis of AS. These methods do help exclude other nephropathies. The renal architecture is preserved in younger patients with AS; a picture of "mixed nephritis" combining interstitial fibrosis and segmental glomerulonephritis develops with increasing age [6]. Foam cells, once considered quite suggestive of AS, are in fact observed in many other glomerular diseases associated with heavy proteinuria [8]. Immune deposits usually are absent; traces of IgM, C3, or both have been observed [8, 24].

By contrast, electron microscopic studies have revealed

characteristic alterations of the GBM [45]. Over the past 10 years, the development of specific antibodies has permitted the detection of each α(IV) chain in the basement membranes of kidney and skin. At present, immunohistochemistry is a new, useful tool for the diagnosis of AS.

Ultrastructure of the GBM. The most characteristic histologic feature of AS is variable thickness of the GBM with lamellation of the lamina densa often circumscribing clear areas containing electron-dense granules (Fig. 2). The GBM width, which normally is 350 nm, can be as thick as 1200 nm or as thin as 100 nm. Its inner and outer contours are irregular; thick and thin segments can alternate. The specificity of these changes has been questioned since segmental areas of GBM splitting have been observed in various immune-complex glomerulonephritides [7]. Furthermore, diffuse thickening of the GBM occurs in diabetic nephropathy [46] and in the nail-patella [30] and Cockayne syndromes [47]. Still, in the absence of the latter disorders, extensive thickening and splitting of the GBM, together with the absence of immune deposits, strongly suggest the diagnosis of AS. This ultrastructural appearance can precede the abnormalities detected by light microscopy, but it is found in only 60% to 90% of patients with AS [48, 49]. In the majority of the remaining patients, the GBM appears uniformly thin; in some others, unspecific lesions or no lesion at all is detected [49]. Thinning can be the first stage of GBM alteration; thus, sequential biopsies in a male patient at ages 9 and 18 years revealed a progression from diffuse attenuation in the first sample to characteristic thickening and lamellation in the second [50]. In some families, X-linked as well as autosomal-recessive, diffuse thinning is the only GBM abnormality, even until adulthood [2, 48]. Diffuse GBM thinning is the hallmark of another clinical entity known as benign familial hematuria. In AS, diffusely thin GBM is often associated with a milder phenotype and less severe mutations: 36% of 25 patients with thin GBM studied by Gubler et al were not deaf versus only 5% of 57 with thick GBM [48]; all seven patients with thin GBM reported by Nakanishi and colleagues had adult-type AS versus only one of 16 with thick GBM [51]. This pattern is not a rule, however; a major rearrangement was recently reported in a patient with no significant lesion in the GBM [49], and discordant ultrastructural findings have been observed among affected relatives in several families [48, 49]. In sum, the ultrastructural pattern correlates, but not strictly, with clinical severity and magnitude of mutation.

α(IV) chains in the kidney. Expression in the normal kidney of the four α(3-4-5-6) chains of type-IV collagen encoded by the genes defective in AS varies among nephron segments. The α3 and α4 chains are expressed in the GBM, Bowman's capsule, and distal tubule, but not in the proximal tubule, Henle's loop, and collecting duct; the α5(IV) chain has the same distribution except for its presence in the collecting duct; the α6(IV) chain co-localizes with the α5(IV) chain except for its notable absence in the GBM (Table 3) [2, 10, 11, 43, 52–56].

Table 3. Typical distribution of the six α(IV) collagen chains in selected renal and skin basement membranes from normal individuals and AS patients (hemizygotes and AR homozygotes)[a]

	α1	α2	α3	α4	α5	α6
Normal						
GBM[b]	+	+	+	+	+	−
Bowman's capsule	+	+	+/−	+/−	+	+
Distal tubule	+	+	+/−	+/−	+	+
Collecting duct	+	+	−	−	+	+
EBM	+	+	−	−	+	+
Hemizygote						
GBM	+	+	−	−	−	−
Bowman's capsule	+	+	−	−	−	−
Distal tubule	+	+	−	−	−	−
Collecting duct	+	+	−	−	−	−
EBM	+	+	−	−	−	−
AR homozygote						
GBM	+	+	−	−	−	−
Bowman's capsule	+	+	−	−	+	+
Distal tubule	+	+	−	−	−	−
Collecting duct	+	+	−	−	+	+
EBM	+	+	−	−	+	ND[c]

[a] From Refs. 2, 10, 11, 43, 52–56
[b] GBM: glomerular basement membrane; EBM: epidermal basement membrane
[c] ND: not determined

The absence of α5(IV) in the GBM was expected in the X-linked AS, whereas that of α3(IV) or α4(IV) was anticipated in the autosomal-recessive form. In fact, GBMs lacking α5(IV) also failed to express the α3(IV) and α4(IV) chains with a few exceptions (Table 3) [2, 7, 11, 52]. In other words, a mutation affecting one of the three chains prevents the expression not only of that chain but also of the other two. Possible explanations for this phenomenon have been discussed elsewhere [7, 9]. It is generally assumed that a specific α3-α4-α5 network exists in the GBM, and that a mutation in one of the three chains prevents the incorporation of the two others into a stable heterotrimer, resulting in their degradation [7, 9]. Whatever the explanation, the co-absence of α3 (always concordant with α4) and α5 in the GBM is a major diagnostic clue to AS, because it has not been described so far in other nephropathies, including minimal-change disease, immune-complex glomerulopathies, diabetic nephropathy, and thin-basement-membrane nephropathy [7, 43, 54, 55]. This finding is especially helpful when the GBM ultrastructural appearance is equivocal. As a result of random X-chromosome inactivation, XL heterozygotes most often express α3 and α5 mosaically [7, 43, 48, 51]; the degree of α5 expression seems to correlate negatively with the severity of renal disease [57].

Although specific, the absence of both α3 and α5 in the GBM is not a sensitive clue for AS. A faint or even normal staining for both α3 and α5 is indeed observed in about 20% of hemizygotes and AR homozygotes

[2, 40, 49]. These patients usually have a milder phenotype [48, 51], diffuse GBM thinning [48, 51], and less severe mutations in the COL4A5 gene (mis-sense and small in-frame deletion/insertion) [49, 58]. In addition, although the expression of α3 and α5 in the GBM is usually concordant, the presence of α3 in the absence of α5 has been reported in seven families [49, 51], two of whom had a COL4A5 mutation, a glycine substitution in the triple helix in both cases [49].

Clearly, the pattern of α3-α5 chains in the GBM does not differ between the X-linked and the autosomal-recessive form. By contrast, the study of α(IV) chains in the extraglomerular basement membranes might help differentiate both forms of AS. In Bowman's capsule and the collecting duct, both α5 and α6 are expressed in autosomal-recessive AS but are absent in the X-linked form (Table 3) [2, 56]. This pattern might be explained by the normal scarcity (in Bowman's capsule) or the absence (in the collecting duct) of α3 and α4 chains at these sites. A mutation in the COL4A3/A4 genes is thus expected to interfere less severely or not at all with α5(IV) expression, as compared with GBM in which α3 and α4 form heterotrimers with α5. This distinction is not absolute, however; a pattern characteristic of the autosomal-recessive form has been reported in a few patients with a splice site of a mis-sense mutation of COL4A5 (Gubler MC, personal communication) [58].

α(IV) chains in the skin. The α5 and α6(IV), but not the α3 and α4(IV), chains are expressed in the epidermal basement membrane (EBM) [4, 51, 53, 55], so biopsy examination of the skin can be valuable in the diagnosis of AS [7].

The expression pattern of α5(IV) is the same in the GBM and EBM from patients with X-linked AS, whether hemizygotes [43, 51] or heterozygotes [57]. A case of discordant pattern (the presence of α5 in the EBM despite its absence in the GBM) nevertheless was reported in two related hemizygotes [59]. Still, the absence of α5(IV) from the EBM appears highly specific for X-linked AS; this finding has not been reported so far in other conditions. Among XL heterozygotes expressing α5 mosaically, the degree of expression—quantified as the percentage of EBM length stained by anti-α5(IV) antibodies—inversely correlates with the severity of renal involvement, as assessed by proteinuria [57]. In AR homozygotes, α5(IV) is always expressed in the EBM, just as in extraglomerular basement membranes within the kidney [2].

Valid immunohistochemical examination of kidney and skin with antibodies against α(IV) chains requires experience with this technique [7]. Staining is generally studied in fresh-frozen sections using the indirect immunofluorescence method. To reveal epitopes, sections have to be treated with 6 M urea prior to exposure to primary antibodies. Parallel staining of control tissues side by side with patient tissues is recommended [7]. Staining of paraffin-embedded sections using the immunoperoxidase method also has been performed successfully, provided the reactive epitopes are previously restored by an antigen retrieval procedure [58]. A recently established fluorochrome-conjugated monoclonal antibody against the triple-helical domain of α5(IV) reportedly is usable in direct staining without urea pre-treatment [60].

Genetic diagnosis

Genetic analysis provides the only conclusive diagnosis of AS. As I already mentioned, direct mutation detection in AS remains a challenge for the molecular biologist because of heterogeneity of the locus (several genes) and allele (many different types of mutation in the same gene) in addition to the huge size of the genes (51 exons to be screened). Until recently, about 50% of mutations affecting the most frequently involved gene, COL4A5, remained undetected [61–63]. Using PCR and direct DNA sequencing of all exons, including extended intron sequences, Martin et al have just recently reported a mutation detection rate of 82% [64]. Nevertheless, until advances are made in speed and yield of the techniques used for genetic mutation screening, we will have to rely on the indirect approach, genetic linkage analysis [65].

Linkage analysis. Linkage of a disease to a genetic locus relies on the demonstration of the co-segregation, in a given family, of the disease with a genetic marker or set of markers (DNA sequences known for their polymorphism) flanking or within the gene. DNA markers mainly include restriction fragment length polymorphisms (RFLP) and sequences containing a variable number of simple repeat elements, such as a dinucleotide tandem repeat (DNTR), for example, the repeating unit CA.

For COL4A5, many markers near or within the gene have been identified [65]. We routinely use 4 DNTR:2B6, which is intragenic; DXS456, which is proximal; and DXS1191 and DXS1106, which are distal to COL4A5, as primary markers. Additional markers are used when primary markers are not sufficiently informative. A smaller number of intragenic and flanking markers for the COL4A3/A4 genes are available [14, 15]. We currently use a CA repeat and a COL4A4 intragenic RFLP [14].

In genetic linkage analysis, the accuracy of the result—expressed as a likelihood of linkage versus non-linkage—depends on several factors: the a priori probability for linkage (based both on the frequency of the tested genetic form in the relevant population and on the individual pedigree), the correct identification of affected or non-affected family members, the number of tested individuals, and the informativeness of the markers. For example, in a small family, linkage to COL4A5 might only be likely or very likely. In that case, the exclusion of linkage to COL4A3/A4 would reinforce the probability of linkage to COL4A5. Potential pitfalls in linkage

data are non-paternity and the occurrence of new mutations, including germ-line mosaïcism [65].

Mutation screening. Only a handful of mutations have been identified in the COL4A3/A4 gene. Therefore, I will only mention the current strategy used to detect mutations in the COL4A5 gene. Only large and medium-sized deletions are easily detected by a simple technique, that is, Southern blotting analysis using overlapping cDNA probes spanning the entire gene. This first step in mutation screening can identify only 2% to 15% of COL4A5 mutations [12, 62, 66]. More sophisticated methods are used subsequently for the diagnosis of other, small mutations. Since there is no "hot spot," the whole gene has to be examined exon by exon. The screening methods have been reviewed by Lemmink et al [12] and Renieri and De Marchi [67]. The most widely used method is the single-strand conformation polymorphism analysis (SSCP) after PCR amplification of each exon; this technique relies on the observation that a denatured DNA sample subjected to migration through a gel matrix has a different conformation, and therefore a different mobility, if its nucleotide sequence is modified. This step is followed by sequencing the abnormal exon [61–63, 67]. Direct DNA sequencing after PCR amplification of all exons is now recommended as the most effective approach [64]. As I already mentioned, many mutations have been recognized; only a few have been recorded more than once in different families.

Use of the genetic diagnosis

Given their complexity and cost, the use of methods currently available for genetic diagnosis is restricted to selected cases. In families with an established diagnosis of AS, genetic diagnosis can clarify the mode of transmission, which is of crucial importance for genetic counseling. Genetic diagnosis also can ascertain the status of an XL heterozygote for the purposes of family planning or kidney donation and provide prenatal diagnosis. Both direct identification of the mutation [68] and gene linkage [69] have been used for prenatal diagnosis of juvenile AS. Detection of mutation also might be the only way to establish the diagnosis of AS in the occasional patient in whom uncertainty remains after extensive clinical evaluation.

Thin GBM nephropathy

As I said earlier, the GBM can be diffusely thin in AS, either as an early change preceding thickening [50] or as the only persistent lesion in some X-linked as well as in some autosomal-recessive [2, 48] kindreds. Thin GBM is also observed in a reportedly different condition called "benign familial hematuria," characterized by isolated, persistent microscopic hematuria without extrarenal abnormalities, transmitted as an autosomal-dominant trait [70].

A regular and diffuse thinning of the GBM, with a mean GBM thickness of less than 260 nm, occurs in patients with benign familial hematuria in the absence of other specific lesions on light and immunofluorescence microscopy [70, 71]. Staining of the GBM for both α3(IV) and α5(IV) is normal or only slightly reduced [60, 70, 72]. An increased percentage of sclerotic glomeruli has been reported in some patients [73]. The prevalence of thin GBM was found to be similar to that of IgA nephropathy among Dutch patients biopsied for chronic hematuria [71]. Recent information suggests first, that benign familial hematuria might not be so "benign" in the long-term, and second, that it could belong, at least for a subset a patients, to the spectrum of AS.

Most patients with a thin GBM only have lifelong microscopic hematuria, but gross hematuria, proteinuria, and renal failure have been occasionally reported [74, 75]. Among 17 normotensive patients with thin GBMs followed for 12 years, seven became hypertensive and one of these developed mild renal failure; second biopsies in three patients revealed unchanged thin GBMs but increased focal glomerulosclerosis [73].

The similarity of GBM abnormality in benign familial hematuria and in early AS together with the frequent presence of microscopic hematuria in AS heterozygotes led to the hypothesis that patients with thin GBM nephropathy were in fact AR heterozygotes; this hypothesis also would account for the dominant transmission of this abnormality [14]. This route of transmission was demonstrated in a Dutch family in which a mis-sense mutation in the collagenous domain of COL4A4 cosegregated with microscopic hematuria [14]. Unfortunately, only the patient suspected of being a compound heterozygote had a renal biopsy, which showed GBM abnormalities typical of AS. By contrast, investigation of four Japanese families with biopsy-proven thin GBM nephropathy failed to show linkage to COL4A3 markers [76] and suggested the implication of other genes. Ongoing studies of similar families in Europe should clarify the intriguing relationship between AS and thin GBM nephropathy.

Autosomal-dominant AS

A rare form of autosomal-dominant, progressive hematuric nephritis associated with deafness, macrothrombocytopenia (Epstein's syndrome), and sometimes granulocyte inclusions (May-Hegglin anomaly or Fechtner's syndrome) has been reported. Gregory et al reviewed data from 13 families [18]. Ultrastructural appearance of the GBM, reported in five cases, is suggestive of AS [18]; the distribution of α(IV) chains, reported in three cases, is normal [77]. In the absence of molecular biologic data, the relationship of this entity to AS remains uncertain.

In a few AS kindreds without hematologic abnormalities, the occurrence of male-to-male transmission has

Table 4. Diagnostic value of clinical and pathologic features in AS

Pathognomonic
Anterior lenticonus
Absent (hemizygotes) or discontinuing staining (XL heterozygotes) for α5(IV) in the EBM[a]
α3 − α4 − α5(IV) in the GBM
Highly suggestive
Retinal flecks
History of recurrent corneal erosion
GBM thickening and splitting
Suggestive
Sensorineural deafness
GBM diffuse thinning

[a] EBM: epidermal basement membrane; GBM: glomerular basement membrane

suggested the existence of an autosomal-dominant form [15, 49, 65]. The likely candidate genes are, of course, the COL4A3/A4 genes, responsible for the autosomal-recessive form of AS. Mutation in another collagen gene, COL11A2, was shown to lead either to recessive or dominant disease, according to the type of mutation [78]. To the best of my knowledge, linkage to the COL4A3/A4 genes has been reported in only one family with autosomal-dominant AS; in this family, AS was of the adult type, and extrarenal involvement was restricted to mild deafness in a single affected individual [15].

Before establishing a diagnosis of autosomal-dominant AS, one should consider the possibility of other autosomal-dominant progressive glomerulonephritides unrelated to AS. One of them, recently recognized, is focal segmental glomerulosclerosis, clinically characterized by long-standing proteinuria before ESRF and the absence of extrarenal abnormalities [79]; this entity is genetically distinct from AS (abstract, Pirson et al, *Nephrol Dial Transplant* 13:A103, 1998).

Diagnostic approach

How should we approach the diagnosis of AS? I will consider first the apparently sporadic patient suspected of having AS in whom the diagnosis and the mode of transmission have yet to be established. Second, I will consider the at-risk individual from a known AS family. I will talk about only the two main forms of AS, namely, X-linked and autosomal-recessive AS.

When should we suspect an apparently sporadic case of AS? Alport's syndrome should be considered in any child with unexplained, persistent microscopic hematuria of glomerular origin, with or without a history of gross hematuria. Alport's syndrome also should be kept in mind in an adult with a clinical or pathologic picture of unspecified chronic glomerulonephritis. The presence of an extrarenal abnormality associated with AS (Table 4) obviously raises the index of suspicion, although its absence does not rule it out.

The clinical workup should include, whenever possible, the screening of first-degree relatives (at least for microscopic hematuria) and, in the proband, a careful eye examination and an audiogram. Isolated microscopic hematuria in the proband's mother is consistent with both forms of AS; its presence in the proband's father suggests an autosomal-recessive form; found in two generations with male-to-male transmission, isolated microscopic hematuria points to a diagnosis of benign familial hematuria.

The diagnostic value of each extrarenal abnormality is summarized in Table 4. Anterior lenticonus (virtually always associated with deafness), the only pathognomonic extrarenal feature, is found in only 10% to 30% of affected patients. In its absence, the likelihood of the diagnosis is very high in the presence of three or even two other characteristic clinical features (Table 4). For example, the combination of retinal flecks and sensorineural deafness strongly predicts AS.

The next step in diagnosing AS relies on pathologic evidence. Skin biopsy is less invasive than renal biopsy and should be performed first. The absence of α5 in the EBM, observed in about 80% of hemizygotes, or the discontinuous pattern characteristic of the corresponding XL heterozygotes (although less easily recognized) is pathognomonic of X-linked AS. Normal staining (either in hemizygotes or XL heterozygotes) does not exclude a diagnosis of X-linked AS.

If the diagnosis is not yet established, renal biopsy most often provides it. Diffuse thickening and splitting of the GBM, observed in 60% to 90% of AS patients, strongly suggests AS. Diffuse thinning, observed in the majority of remaining affected individuals, is compatible with either AS (early stage or not) or thin GBM disease. Normal ultrastructure of the GBM makes the diagnosis of AS very unlikely. The interpretation of staining for α3-α5(IV) in the kidney is summarized in Table 3. Again, normal staining, found in 15% to 20% of AS patients, does not exclude the diagnosis of AS. If the diagnosis remains doubtful (and sophisticated genetic laboratory testing is available), one could consider screening for a genetic mutation. Screening should be started with the COL4A5 gene, unless a recessive form is suspected.

Screening of first-degree relatives is critical. The following findings suggest an autosomal-recessive form: consanguinity of the parents, no abnormality or only mild abnormalities in the parents (most often microscopic hematuria, sometimes mild hearing loss), or severe disease of a female proband with ESRD before the age of 20. The autosomal recessive form differs from the typical X-linked form by the presence of α5(IV) chain, both in some extraglomerular basement membranes and in the EBM (Table 3). The existence of a few exceptions should be kept in mind.

How should we confirm the diagnosis of AS in an at-risk member of a family with AS? In a family with X-

linked AS, early diagnosis among at-risk males (that is, 50% of the children from a heterozygote mother) generally is easy; persistent microscopic hematuria renders the diagnosis of AS very likely (provided that urologic causes have been ruled out), whereas its absence virtually excludes it. The presence of one extrarenal sign of the disease, including EBM immunostaining for α5(IV), is confirmatory (Table 4). In at-risk females without renal or extrarenal signs of AS, linkage analysis is the most reliable method of formally excluding the genetic diagnosis of asymptomatic carrier.

In a family with autosomal-recessive AS, normal urinalysis excludes homozygosity; microscopic hematuria is found in all homozygotes and in about one-half of the heterozygotes. Severe renal and extrarenal involvement is only found in homozygotes. If the diagnosis remains doubtful, linkage analysis should be performed.

Conclusion

The history of Alport's syndrome illustrates the impact of molecular biology on our comprehension and classification of renal disorders. Described more than 60 years ago as a syndrome associating hereditary renal disease and deafness [80], AS became a family of diseases with blurred outlines. The discovery of its molecular determinants has provided a diagnostic gold standard and given us an understanding of the various components of the clinical syndrome. A disease of type-IV collagen, AS encompasses several varieties defined by the corresponding defective gene: X-linked disease due to mutation in the COL4A5 gene, X-linked disease with diffuse leiomyomatosis due to contiguous mutation in the COL4A5 and COL4A6 genes, and autosomal-recessive disease due to mutation either in the COL4A3 or the COL4A4 gene. For each of these varieties, the discriminant value of associated clinical findings, family history, ultrastructural aspect of the GBM and, more recently, the staining pattern for α(IV) chains in kidney and skin basement membranes has been evaluated, so that the clinician is now able to confidently establish the diagnosis of AS in a majority of patients on clinical and pathologic grounds. In the remaining cases, in which the diagnosis remains uncertain, genetic techniques may provide the ultimate answer.

Despite this impressive progress, the diagnosis remains elusive in a few cases, and the borders between autosomal-recessive AS, benign familial hematuria, and autosomal-dominant AS are yet to be delineated. Other components of the GBM, such as laminin, nidogen, and proteoglycans might be involved. Further elucidation of the molecular basis of the various autosomal varieties of AS will undoubtedly widen our understanding of hereditary nephropathies.

QUESTIONS AND ANSWERS

Dr. John T. Harrington (*Dean, Tufts University School of Medicine, Boston, Massachusetts, USA*): Thank you, Yves, for a superb review of the genetic profile of Alport's syndrome. Is there any information regarding genetic alterations in type-IV collagen in patients with GBM thickening due to a systemic disease such as diabetes? Might such alterations play a contributory role in that disorder?

Dr. Pirson: I am not aware of the existence of specific genetic alterations in type-IV collagen in diabetics developing GBM thickening. But it is noteworthy that the composition of the thickened GBM differs between the two conditions. In AS, α1(IV) and α2(IV) chains, normally confined to the subendothelial region of GBM, extend throughout the full width of GBM, along with type-V and type-VI collagens [7]. Such changes are not present in diabetic nephropathy [81].

Dr. Harrington: Has gene replacement therapy been explored in animal models? Do you foresee any role for gene therapy in humans, and what are the obstacles?

Dr. Pirson: Four different animal models of AS are available, three in dogs and one generated in mutant mice. In the Samoyed dog model, which has been thoroughly characterized, AS is X-linked and due to a single base mutation in the COL4A5 gene producing a premature stop codon [82]. Two models exist for the autosomal recessive form: a COL4A3 knockout mouse [83] and a naturally occurring disease in the English cocker spaniel dog [84]. Last, an autosomal-dominant form has been reported in the bull terrier dog [85].

Alport's syndrome is a conceptually attractive disease for testing the potential of gene therapy. The fact that XL as well as AR heterozygotes usually do not develop renal failure indeed suggests that provision of a normal copy of the gene could prevent progressive renal disease. This has yet to be demonstrated in animal models. The obstacles to successful gene therapy are numerous, ranging from the difficulty of effectively targeting the appropriate kidney cells, to the problems related to the control of the expression of the transferred gene so as to obtain a correct incorporation of the collagen chain into GBM [86]. As an encouraging first step forward, Tryggvason and colleagues recently reported 85% transfer efficiency of a reporter gene carried by an adenovirus vector into *in vivo* perfused pig glomeruli [86]. Prior to the advent of gene therapy, animal models will help us to test the effect of pharmacologic interventions such as ACE inhibitors.

Dr. Jerome P. Kassirer (*Editor-in-Chief*, New England Journal of Medicine*, Boston, Massachusetts, USA*): Aside from the intellectual satisfaction, are there any therapeutic implications of a specific histologic and genetic diagnosis of the type of Alport syndrome?

Dr. Pirson: So far the diagnosis of AS has no direct therapeutic impact, narrowly defined. Nevertheless, recognition of the disease and identification of its mode of transmission have significant implications in terms of prognosis and genetic counseling of the patient and the extended family.

Sir Keith Peters (*Regius Professor of Physics, University of Cambridge, Cambridge, UK*): As your presentation indicated, precise clinical and genetic characterization also has important implications for living-related organ donation, as well as for the risk of subsequent anti-GBM disease in the graft.

Dr. Pirson: You rightly emphasize that ascertaining the status of XL or AR heterozygotes by linkage analysis or identification of the mutation has important implications for kidney donation. The type of mutation also has an impact, although modest, on the risk of developing anti-GBM disease after renal transplantation. Let me first stress that the risk of graft loss from anti-GBM disease often has been exaggerated. In our experience with more than 50 patients who received renal transplants for AS, no graft has been lost so far from anti-GBM disease [87], despite the fact that in about 15% of the grafts the presence of a linear glomerular deposit of IgG reflects a mild immunization against GBM [87, 88]. The clinical and genetic profile of the 3% of AS patients who develop crescentic anti-GBM nephritis with eventual graft loss has been recently reviewed by Kashtan and Michael [7] and Lemmink and colleagues [12]. Roughly, 90% are males with a juvenile-type XL disease and 10% are AR homozygotes. Nearly all patients have a mutation resulting in a truncated α(IV) chain lacking the NC1 domain. Still, the majority of patients with a mutation deleting a large part of COL4A5, COL4A3, or COL4A4 (including the NC1 domain) do not develop anti-GBM nephritis. Thus, factors other than the type of mutation must influence the immune response to the graft. In practice, the discovery of such a mutation should not contraindicate renal transplantation [7, 12, 66]. Of note, the target of antibodies developed in hemizygotes against graft GBM is generally, as expected, the NC1 domain of α5(IV) [89]; such antibodies are therefore not recognized by commercial ELISAs for anti-GBM antibodies in which the ligand is a purified preparation of α3(IV) NC1 domains (the Goodpasture antigen).

Dr. Christopher Winearls (*Chief, Renal Unit, Oxford Radcliffe Hospital, Oxford, UK*): Do pregnancies affect the renal prognosis of X-linked Alport syndrome patients?

Dr. Pirson: I am not aware of any study on the effect of pregnancy on the renal outcome of XL heterozygotes.

Dr. Jacques Bernheim (*Chief, Renal Unit, Meir Hospital, Kfar Saba, Israel*): You mentioned the Epstein syndrome briefly. Do you think that this disease really is a separate entity?

Dr. Pirson: This syndrome differs from classic AS by its autosomal-dominant inheritance and the normal expression of α(IV) chains in the GBM [18, 77]. Only the identification of the molecular basis of this disorder will reveal whether it is simply a variant of AS or a separate entity.

Dr. Toshio Miyata (*Associate Professor of Medicine, Tokai University School of Medicine, Kanagawa, Japan*): You ascribed the wide range of severity of AS observed in XL heterozygotes to the random inactivation of the X chromosome. We also found the effect of random inactivation of the X chromosome in paroxysmal nocturnal hemoglobulin (PNH), which is an acquired hematologic disorder. Paroxysmal nocturnal hemoglobulinuria is caused by the somatic mutation of PIG-A gene on X chromosome [90]. However, the incidence of PNH is equal between males and females because of random inactivation of the X chromosome. Therefore, I agree that the random inactivation mechanism of the X chromosome functions in the pathology. Could you comment on the random inactivation of the X chromosome in XL heterozygotes with AS? Do one or more nongenetic mechanisms account for the wide range of severity of AS in XL heterozygotes?

Dr. Pirson: Let me first remind us that the variable severity of the expression of an X-linked disorder in heterozygotes can depend both on genetic factors, that is, the type of mutation and the modifying genes, and non-genetic factors. However, a major determinant of this variability seems to be the individual pattern of X inactivation. As you know, the inactivation of one of the two X chromosomes takes place very early during embryogenesis and is random between the maternal and paternal X chromosomes. As a result, most females have a mosaic expression of maternal and paternal alleles of X loci, with a mean contribution, at a population level, of 50% from each chromosome. However, a substantial deviation from this 50:50 population distribution, or "skewing," is observed in 5% to 20% of individual women [91]. Preponderant inactivation of the normal X chromosome therefore could account for a severe phenotype in XL heterozygotes, such as in the patient reported by Guo et al, in whom 90% of the X chromosomes carrying the normal COL4A5 allele were inactivated, both in kidney and white blood cells [92]. The elucidation of the processes leading to X inactivation skewing should enlighten our understanding of the phenotypic heterogeneity among XL heterozygotes.

Dr. Jean-Pierre Grünfeld (*Chief, Renal Unit, Hôpital Necker, Paris, France*): We know that approximately 12% of the heterozygote women with X-linked AS progress to renal failure. In your opinion, what are the best predictors of progression in a 20-year-old affected woman?

Dr. Pirson: The strongest predictors remain those that

you identified more than 10 years ago: a history of gross hematuria, the nephrotic syndrome, and a diffuse thickening of the GBM [19]. An additional parameter could be the degree of (α5)IV expression, measured either in the GBM or the EBM. Nakanishi has indeed reported that this expression is inversely correlated with the severity of renal involvement [57]. If this observation is confirmed, quantification of (α5)IV expression in the EBM could become a useful predictor of renal outcome in XL heterozygotes.

Dr. Tilman Drüeke (*Director of Research, INSERM U90, Hôpital Necker*): In the mouse knockout model of autosomal-recessive AS, is the glomerular basement membrane either thin or thick or both? Is there a progression from thin to thickened membrane with time?

Dr. Pirson: The COL4A3 knockout mouse is an excellent model for following the course of GBM alterations. As in human disease, there is clearly a progression from GBM thinning, beginning in the external capillary loops, to extensive thickening and lamellation. As in human disease also, GBM lacks α4(IV) and α5(IV) chains, although mRNAs encoding these chains normally are present [83]. Similar observations have been made in the dog model of autosomal-recessive AS [84]. Both in the mouse and dog models, heterozygotes exhibit a normal GBM appearance [83, 84].

Dr. Marie-Claire Gubler (*Director of Research, INSERM Unit 423, Hôpital Necker*): In your diagnostic strategy, you consider immunohistochemical changes of the GBM or EBM as pathognomonic of AS. I agree with you. I feel that this feature has become a definitive criterion for AS. I also want to emphasize that ultrastructural findings also have a high diagnostic value when they are observed in the absence of other pathologic changes and when they are widespread, for example, in a child with isolated hematuria.

Dr. Pirson: I agree. Thank you for your comment.

Dr. Charles van Ypersele (*Professor Emeritus, Renal Unit, Cliniques Universitaires St-Luc, Brussels, Belgium*): This patient was transplanted with her mother's kidney. This is easy to understand because the diagnosis of AS was not known at that time. What would be your attitude today if you knew the diagnosis?

Dr. Pirson: The vast majority of AR heterozygotes will remain asymptomatic carriers. In those without microscopic hematuria, there is no reported case of progression to renal failure; kidney donation thus should be accepted without hesitation. Those AR heterozygotes who have isolated microscopic hematuria have a very small risk of developing late renal failure. In that case, I would express some reservation about kidney donation but would be ready to accept it from a strongly motivated, well-informed donor. Rare heterozygotes have additional manifestations such as proteinuria, hypertension, or sensorineural deafness. They should not be accepted as kidney donors.

Dr. Joseph Rosenfeld (*Chief, Institute for Preventive Cardiology, Tel Aviv University Medical School, Tel Aviv, Israel*): Following Charles van Ypersele's question, I would like to add that living-related donor transplantation in an AS patient has an excellent prognosis. Our first such patient received a kidney from his mother. He survived for 33 years with an excellent creatinine clearance and eventually died from chronic hepatitis.

Dr. Harrington: Could you speculate further on the relationship between thin-basement-membrane (TBM) disease and AS? Will nearly all TBM disease patients turn out to have AS?

Dr. Pirson: We should first be aware that in the literature there is a problem of definition of thin GBM, because pathologists rely on different cut-off points and different examination methods. As we have just discussed, thin GBM can be the temporary or, more rarely, the persistent expression of a true AS, that is, a disease that will progress to glomerulosclerosis and renal failure. Thin GBM also can be the sole pathologic counterpart of so-called benign familial hematuria, a subset of which might be—at least in Japan—unrelated to type-IV collagen genes [76]. This latter finding is consistent with the loss of anionic charge in the GBM documented by Goel et al in patients with ultrathin GBM and attributed to an alteration of proteoglycans [93]. My guess is that TBM will be divided into subentities according to the involved GBM component. Marie-Claire, you are the best expert in the field. What do you think?

Dr. Gubler: Indeed I do believe that thin GBM is not a disease but only an ultrastructural finding that may correspond to various types of nephropathy. As a pathologist, I can identify the lesion, but not necessarily the disease.

Dr. Jürg Schifferli (*Professor of Medicine, University Hospital Basel, Basel, Switzerland*): What is so special about type-IV collagen that it is included in the basement membranes of kidney, eye, and ear? Has it a specific property or function?

Dr. Pirson: Type-IV collagen is the major component of basement membranes and is almost exclusively found in these structures. While α1(IV) and α2(IV) are present in all basement membranes, α3–α6(IV) have a restricted tissue distribution [10]. The latter, however, are not confined to basement membranes of kidney, eye, and ear; they also are expressed, for example, in brain, lung, and synaptic muscle fibers [10]. The specific biologic role of type-IV collagen has yet to be elucidated. *In vitro* studies have shown that type-IV collagen has the ability to bind integrins and to adhere to a variety of cells [10].

Dr. Mohamed Ben Hmida (*Assistant Professor, Department of Nephrology, Sfax Hospital, Sfax, Tunisia*): What is the mechanism of chronic renal failure in AS?

Dr. Pirson: The mechanism of progressive renal sclerosis in AS has yet to be elucidated. As proposed by Kashtan and Michael [7], accumulation of α1(IV) and α2(IV) chains and subsequently of type-V and -VI collagens in the GBM could disrupt its filtration properties and explain proteinuria. Kalluri et al recently demonstrated that GBMs lacking α3(IV) and α4(IV) chains are less resistant to endopeptidase digestion [94]. Here, too, observation of animal models of AS could provide some clues to the understanding of the mechanisms of chronic renal failure.

NOTE ADDED IN PROOF

Boye et al recently reported the complete characterization of the 48 exons of the COL4A4 gene and described 10 novel mutations in 8 AR homozygotes; they also showed that AR heterozygotes may have microscopic hematuria and thin basement membranes in keeping with Lemmink's observation [14] [Boye E, Mollet G, Forestier L, Cohen-Solal L, Heidet L, Cochat P, Grunfeld JP, Palcoux JB, Gubler MC, Antignac C: Determination of the genomic structure of the COL4A4 gene and of novel mutations causing autosomal recessive Alport syndrome. *Am J Human Genet* 63:1329–1340, 1998].

Van der Loop et al confirmed that the absence of COL4A5 (total in males, focal in females) in the EBM of skin biopsy specimens can be used for identification of XL AS [Van der Loop FTL, Monnens LAH, Schroder CH, Lemmink HH, Breuning MH, Timmer EDJ, Smeets HJM: Identification of COL4A5 defects in Alport's syndrome by immunohistochemistry of skin. *Kidney Int* 55:1217–1224, 1999; Kashtan CE: Alport syndrome: Is diagnosis only skin deep? *Kidney Int* 55:1575–1576, 1999].

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